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NOVEL GENE AND PGTH PROTEIN ENCODED THEREBY

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Field of the technology

This invention pertains to a novel PGTH protein of human brain origin having a prostaglandin transport activity and the pgth gene encoding the protein.

Prior art

Prostaglandin is a generic name for a series of physiologically active lipids such as prostaglandin E, prostaglandin D, prostaglandin F, prostaglandin I, prostaglandin J, etc. Prostaglandin is a physiologically active substance inside the body strongly related to control of physiological functions such as blood flow rate, sleeping, gastric mucosa protective action, thrombus formation, pregnancy, etc., through specific cell membrane or intranuclear receptors.

Prostaglandin is produced inside cells as a result of eicosapolyenic acids such as arachidonic acid, etc., being cut out by phospholipase A2 from the cell membrane and converted with cyclooxygenase and various prostaglandin synthetic enzymes by responding to various physiological stimuli, and after being released outside the cells, it has autocrine or paracrine effects. On the other hand, liberated prostaglandin is also circulated in the blood flow, taken up by a specific cell, metabolized and so disappears.

A trace amount of prostaglandin shows a strong physiological activity, and consequently, the production of prostaglandin compounds is strictly controlled by controlling the activity of production-related and metabolism-related enzymes.

However, prostaglandin has been reported to be unable to pass through the lipid double layer of the cell membrane by itself. Therefore, as a prostaglandin transport mechanism, the presence of a special protein has been presumed in the process of prostaglandin produced inside a cell exiting it and the process of prostaglandin circulating in the blood flow being taken up into a specific cell.

As a protein involved in the transport mechanism described above, prostaglandin transporter (abbreviated hPGT: human prostaglandin transporter, below) has been reported, but it is not a protein involved in the transport of all prostaglandin compounds, and there are many unclear points. Consequently, it is thought that if a biological molecule other than hPGT involved in the transport mechanism can be elucidated, the biological molecule found might be usable directly as a medical drug or indirectly as a compound for studying compounds that might be usable as a medical drug. Therefore, the objective of this invention is to identify such a molecule and use it as a medical drug or for the development of medical drugs.

Presentation of the invention

The inventors of this invention studied diligently to find the desired protein by using genes expressed in the human brain, and as a result, they found the presence of a novel PGTH protein (prostaglandin transporter homologue), successfully isolated a pgth gene encoding the protein, and they arrived at this invention.

Specifically, this invention pertains to (a) a protein having the amino acid sequence described in sequence No. 1 or (b) a protein having an amino acid sequence with 1 to several amino acids deleted, substituted or added to the amino acid sequence of sequence No. 1, and having a prostaglandin transport activity.

Furthermore, this invention also pertains to (c) a gene comprising DNA described sequence No. 2 or (d) DNA which can be hybridized with the DNA of sequence No. 2 under stringent conditions and which encodes a protein having a prostaglandin transport activity.

The pgth gene of this invention can be isolated as a cDNA fragment containing the gene from a cDNA library of human brain origin. The cDNA library used by the inventors of this invention was prepared based on commercially available mRNA of human brain origin from the Clonetech Co.

As a method for identifying the cDNA encoding a protein having a prostaglandin transport activity in the cDNA library described above, the method of Ohara, et al., (DNA Research 4: p 53, 1997) was used as an extensive cDNA library analysis method using a long-chain cDNA library. From a long-chain cDNA library of human brain origin prepared by the method of Ohara, et al., 25,000 recombinants are randomly selected, the 5' and 3' - base sequences of the cDNA from 15,000 clones were determined, and a clone showing homology to the gene encoding hPGT already reported from the 5' sequences of all the clones can be found by using a DNA analysis program (BLAST and FastA).

The presence of a region encoding the protein (ORF: open reading frame) in the base sequence can be confirmed by a conventional method using a computer program. After becoming confident of the presence of the desired gene in the cDNA sequence, the inventors of this invention found one ORF in the sequence by utilizing a computer, the gene was named pgth, and the protein encoded by the gene was named PGTH. The PGTH of the invention is a protein comprising a total of 709 amino acid residues and having a molecular weight of about 80 kd.

The invention pgth is a gene comprising 2130 bp shown in sequence No. 2. By using this pgth and conventional genetic recombination techniques using a suitable host vector system, it is possible to prepare a recombinant gene. As a suitable vector, there are plasmids of *E. coli* origin (such as pBR322, pUC118, etc.), of *Bacillus subtilis* origin, (such as pSH19, etc.) yeast origin plasmid (such as pUB110, pC194, etc.), bacteriophages, animal viruses such as retroviruses,

vaccinia virus, etc., etc. At the time of recombination, it is possible to add translation initiation and termination codons using suitable DNA adaptors. Furthermore, for gene expression, a suitable expression promoter is attached upstream of the gene. The promoter to be used is suitably selected depending on the host used. For example, if the host is *E. coli*, there are T7, lac, trp, λPL promoters, etc.; if the host is a *Bacillus*, there are SPO promoters, etc.; if the host is a yeast, there are PHO5, GAP, ADH promoters, etc.; and if the host is an animal cell, there are SV40-origin, retrovirus promoters, etc.

Furthermore, the gene may be expressed as a fused protein with another protein (such as glutathione-S-transferase, protein A, etc.). In the case of a fused PGTH prepared by using such a method, a suitable protease (such as thrombin, etc.), may be used to cut out the protein.

As a host usable in the case of PGTH expression, there are various strains of *Escherichia coli*, various strains of *Bacillus subtilis*, various strains of the yeast *Saccharomyces cerevisiae* and animal cells such as COS-7, CHO cells, etc.

As a method for transforming a host cell using the above recombinant vector, a specific method conventionally used to transform the selected host cell is used.

Incidentally, in this invention, DNA which has a DNA sequence other than that shown in sequence No. 2 which can be hybridized with the DNA and encodes a protein having a prostaglandin transport activity, is also included in the scope of this invention.

Specifically, DNA which has a DNA sequence, the total length of the pgth sequence, partially changed due to various artificial treatments such as random mutations, introduction of site-specific mutations, or mutagen treatment, DNA fragment mutation, deletion ligation after scission with restriction enzymes, is also included in the scope of this invention in spite of having a DNA sequence different from that of sequence No. 2 as long as such a DNA variant can be hybridized with pgth under stringent conditions and encodes a protein having a prostaglandin transport activity.

The extent of the above DNA mutation is within the allowable range if the variant has 90% or higher homology with the DNA sequence of pgth. Furthermore, as an extent of hybridization with pgth, Southern hybridization with pgth may be carried out under conventional conditions, for example, in the case of probe labeling with a DIG DNA Labeling kit (Boehringer-Mannheim Cat. No. 1175033), hybridization conditions of a DIG Easy Hyb solution (Boehringer-Mannheim Cat. No. 1603558) at 32°C and washing of the membrane in a 5X SSC solution (containing 0.1% w/v SDS) at 50°C (1X SSC comprises 0.15M NaCl and 0.015M sodium citrate).

Furthermore, a protein encoded by the gene variant which is highly homologous to pgth as described above and has a prostaglandin transport activity is also included in the scope of this invention.

Specifically, a variant having one or more amino acids deleted, substituted or added to the amino acid sequence of PGTH is included in the scope of this invention as long as this variant is a protein having a prostaglandin transport activity.

The side chains of the amino acids, which are the constituent elements of proteins are respectively different with respect to hydrophobicity, electrical charge, size, etc., but several highly conservative relationships in the meaning of practically not affecting the three-dimensional structure (it is also called the steric structure) of proteins have been known from experiences or actual physicochemical observations. For example, for substitution of amino acid residues, there are glycine (Gly) and proline (Pro), Gly and alanine (Ala) or valine (Val), leucine (Leu) and isoleucine (Ile), glutamic acid (Glu) and glutamine (Gln), aspartic acid (Asp) and asparagine (Asn), cysteine (Cys) and threonine (Thr), Thr and serine (Ser) or Ala, lysine (Lys) and arginine (Arg), etc.

Therefore, any variant protein due to substitution, insertion, deletion, etc., in the amino acid sequence of the PGTH shown in sequence No. 1 can be said to be within the scope of this invention if the variation is a variation which conserves the three-dimensional structure of the PGTH, and the protein is a protein having a prostaglandin transport activity similar to PGTH. The allowable extent of this variation is 90% or higher homology with the amino acid sequence shown in sequence No. 1.

Industrial application field

The abnormal expression of pgth or functional failure of PGTH is presumed to be a critical disorder because PGTH has a prostaglandin transport activity, and consequently the normal prostaglandin production mechanism of the body is lost.

Therefore, PGTH itself is considered to be useful as a drug, and on the other hand, pgth or PGTH may be used for effectively studying or evaluating a substance having the same function as that of PGTH, a substance promoting or inhibiting its function, a substance promoting the expression of the gene, etc.

Best embodiment of the present invention

This invention is explained further in detail using application examples as follows, but this invention is certainly not limited to these application examples. Incidentally, unless specified, the experimental procedures used in the following application examples are those

described in standard experimental manuals such as Molecular Cloning, 2nd ed. (Cold Spring Harbor Laboratory Press, 1989), etc., and the operating manuals in commercially available kits, and they can be carried out under the conditions recommended for the respective commercially available products such as restriction enzymes, etc.

Application Example 1 Cloning of pgth

1) Construction of a long chain cDNA library of human brain origin

An oligonucleotide (GACTAGTTCTAGATCGCGAGCGGCCGCCC$(T)_{15}$) containing a NotI site was synthesized using a DNA synthesizer (ABI380B). It was used as a primer, and a double chain cDNA was synthesized using mRNA of human brain origin as a template and the SuperScript II reverse transcriptase kit (Gibco BRL). The ligation of the synthetic DNA was carried out with the cDNA and SalI site-containing adapter (Takara Shuzo), subsequently, NotI digestion was carried out, and cDNA fragments of 3 kb or larger were purified using electrophoresis with a 1% concentration of low-melting agarose.

After ligation of the purified cDNA fragments with a SalI-NotI restriction enzyme-treated pBluescriptIISK+ plasmid, the recombinant plasmids were introduced into *E. coli* ElectroMax DH10B strain (Gibco BRL) using the electroporation method. Subsequently, 25,000 recombinants were randomly selected from the library, the recombinant DNAs were extracted, and the 5'- and 3'-base sequences of the cDNAs of 15,000 clones were determined. For the sequence determination, a PE Applied Biosystem Co., DNA sequencer (ABI PRISM377) and the reaction kit from the same company were used.

2) Selection of clones containing the pgth sequence

The 5 sequences of all the clones determined in 1) were compared with the sequence of hPGT already reported using DNA analytical programs (BLAST and FastA), and as a result, a clone named HK07457 showed significant homology.

3) DNA fragment base sequence determination

The base sequence determination was carried out using a PE Applied Biosystem Co. DNA sequencer and the dye primer method. The sequence was mostly determined using the shotgun method, and for a portion of the base sequence, an oligonucleotide was synthesized based on the base sequence already determined, and the primer walking method was used to determine the entire base sequences of the two chains. The entire base sequence of the cDNA of the clone is shown in sequence No. 3.

The cDNA contains an ORF encoding a protein (PGTH) comprising 709 residues. A termination codon was found to appear in the upstream region of a methionine residue, which was an initiation codon of the protein, with the same reading frame. Therefore, the amino acid sequence shown in sequence No. 3 was confirmed to be the only possibility as an amino acid sequence of the protein encoded by the cDNA fragment.

Figure 1 shows the amino acid homology between already reported hPGT and the PGTH of this invention. The two show high homology, especially, the position of the cysteine residue present at the C-terminal of PGTH is preserved, and the 77^{th} residue glutamine, 561^{st} residue arginine and 614^{th} residue lysine of hPGT, which are amino acids especially important for the transport activity, are also preserved in PGTH.

Application Example 2

Confirmation of protein expression by in vitro translation of pgth

The plasmid containing pgth prepared in Application Example 1 was treated with RNase A, subsequently, RNase A was removed using ADVAMAX beads (AGTC Co.), and in vitro translation was carried out using a TNT T7 coupled reticulocyte lysate system (Promega Co.) in the presence of (^{35}S)-methionine. A portion of the reaction mixture was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the analysis carried out using BAS-2000 (Fuji Shashin Kogyo). As a result, the presence of a single band at about 80 kd was confirmed as shown in Figure 2.

Application Example 3

Construction of animal cell expression vector

1) Amplification of ORF-containing cDNA

An oligonucleotide (following sequence 1) having a sequence upstream from the initiation codon of the protein of sequence No. 3 and oligonucleotide (following sequence 2) having a sequence of a portion downstream from the termination codon of the protein and the reverse complementary strand chain were synthesized using a DNA synthesizer (ABI Co., Model 380B).

Sequence 1

5-CTGGAGCTCACTGCACTCCAGCAGTC-3

Sequence 2

5-AGCTCACACTCGGGAATCCTCTGGCTTC-3

The recombinant cDNA containing sequence No. 3 isolated in application example 1 was used as a template, the oligonucleotides of the sequences 1 and sequences 2 were used as a primer, and the following PCR procedures were carried out using a Takara LA PCR kit Ver. 2 and the PCR thermal cycler MP (Takara Shuzo).

cDNA	5 μL (10 ng)
10X PCR buffer (containing 25 mM Mg^{++})	5 μL
2.5 mM dNTP	8 μL
10 μM Sequence 1	2 μL
10 μM Sequence 2	2 μL
Water	27.5 μL
LA Taq polymerase	0.5 μL
Total amount	50 μL

The PCR cycle was carried out by holding at 94°C for 2 mn, carrying out the reaction at 98°C for 20 sec, cooling to 68°C at a rate of 1°C/2 sec, holding at 68°C for 3 min, at 72°C for 10 min, and repeating 30 times.

The above method was used to amplify a DNA fragment (about 2.2 kb) having a portion of sequence No. 3.

2) Subcloning to an animal cell expression vector

The DNA fragment amplified in 1) was fractionated by 1% agarose gel electrophoresis. After staining the gel with ethidium bromide, the gel containing the desired band observed under ultraviolet irradiation was cut out. The extraction of the DNA fragment from the agarose gel and purification were carried out using a GENECLEAN II Kit (Bio101 Co.)

The extracted and purified DNA fragment was subcloned to animal cell expression vector pTARGET (Promega Co.) The ligation solution used was a Takara Ligation Kit Ver. 2 (Takara Shuzo), and the reaction was carried out with the following composition at 16°C for 1.5 h.

Extracted and purified DNA fragment	1 μL (50 ng)
PTARGET	1 μL (10 ng)
Water	3 μL
Ligation solution	5 μL
Total	10 μL

The reaction solution after the above reaction was used to transform the *E. coli* K12 strain DH5. The transformant was inoculated on an LB agar medium containing 50 μg/mL of ampicillin (Amp), 40 μg/mL of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (IPTG) [sic; isopropyl-β-D-thioglucopyranoside] and 100 μM of isopropyl-b-D-thiogalactopyranoside

(X-gal) [sic; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside] *and cultivated overnight at 37°C.

Each colony that developed on the above plate was inoculated in 10 mL of an LB liquid medium containing 50 μg/mL of Amp, cultivation was carried out overnight at 37°C, the biomass was collected by centrifugation, and subsequently the recombinant DNA was purified using a QIAprep Spin Plasmid Miniprep Kit (Qiagen Co.) to obtain pTARGETpgth.

3) Determination of the base sequence of the inserted cDNA

The base sequence determination was carried out using a DNA sequencer (ABI Co., Model PRISM377) and the dye terminator method, and the whole base sequence of the two chains was determined using the primer walking method. The clone was found to contain all of the region between sequences 1 and 2 among sequence No. 3 confirming that the desired gene pTARGETpgth had been cloned.

Application Example 4

Insertion into CHOk1 cells and stable transformant preparation

The recombinant DNA, pTARGETpgth, prepared in Application Example 2 has a CMV promoter upstream of pgth, and if it is inserted into an animal cell, the expression of pgth is possible.

CHOk1 cells were cultured in 60 mm diameter plastic Petri dishes. As the culture medium, Ham F-12 (Gibco, called growth medium, below) containing 10% fetal bovine serum (Dainippon Seiyaku), 50 U/mL of penicillin and 50 μg/mL of streptomycin was used, and culture was carried out at 37°C in the presence of 5% CO_2. When the cell density was 50%, LIPOFECTAMINE reagent (Gibco) containing pTARGETpgth prepared in Application Example 2 was added in a layer over the cells, incubated for 6 h, and, after replacement with the growth medium, culture was continued for 48 h. After dispersing the cells with trypsin, the cell suspension was placed in a 60 mm diameter plastic Petri dish, and culture was carried out for 24 h. After removing the culture medium, it was replaced by growth medium containing G418 (Gibco, final concentration of 500 μg/mL). The G418 medium was changed every 3 days and culture continued for 2 weeks. When the cell colonies were observable with the naked eye, 3 colonies were isolated using stainless steel cups. As a control, only the pTARGET vector (Promega Co.) was inserted into CHOk1 cells by carrying out the same procedures as those described above to isolate a stable transformant.

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* [Editor's note: The compound names and abbreviations are so garbled in the original text that it is impossible to be certain whether it should be 40 μg/mL IPTG and 100 μm X-gal, or vice-versa.]

washed with a suitable buffer solution containing bovine serum albumin, and culture was continued for 20 min using a buffer solution containing (^{3}H)-labeled PGE2 (Amersham Co.). After washing the cells, they were recovered, and the radioactivity taken up was measured. As a result, the prostaglandin transport activity of the CHOk1 cells with pgth inserted was statistically significantly higher than that of the CHOk1 cells with only the control vector inserted.

Application Example 6

Expression of pgth mRNA in human macrophages loaded with oxidized LDL

1) Preparation of human macrophages loaded with oxidized LDL and normal monocyte cDNA

Normal monocyte cDNA was prepared using RNA prepared with Trizol (Gibco BRL Co.) from CD14-positive monocytes from human peripheral blood as a template and the SuperScript II reverse transcriptase kit (Gibco BRL). Human macrophages loaded with oxidized LDL were prepared by culturing normal monocytes in a RPMI-1640 medium (Dainippon Seiyaku) containing 20% AB serum and antibiotics for 14 days, adding human LDL oxidized with copper sulfate using conventional procedures (oxidized LDL) in the final concentration of 40 μ/mL [sic; dimension incorrect] and continuing culture for 24 h. A method similar to that used for normal monocytes was used to prepare cDNA.

2) Confirmation of pgth mRNA expression by the RT-PCR method

Oligonucleotides (following sequence 3) having a sequence contained in sequence No. 2 and oligonucleotides (following sequence 4) having the sequence of the reverse complementary strand were respectively synthesized using a DNA synthesizer (ABI Co., Model 380B).

Sequence 3

5-GCTCCTGCCCATTGGACGGCTTTAACC-3

Sequence 4

5-TCACACTCGGGAATCCTCTGGCTTC-3

The cDNA prepared in (1) was used as a template, the oligonucleotides with sequences 3 and 4 were used as primers, and the following PCR procedures were carried out using a Takara LA PCR kit Ver. 2 and the PCR thermal cycler MP (Takara Shuzo).

cDNA	2 μL (40 ng)
10X PCR buffer (containing 25 mM Mg^{++})	1.5 μL
2.5 mM dNTP	2.4 μL

10 μM Sequence 3	0.4 μL
10 μM Sequence 4	0.4 μL
Water	10.15 μL
LA Taq polymerase	0.15 μL
Total amount	15 μL

The PCR cycle was carried out by holding at 94°C for 5 min, carrying out the reaction at 94°C for 1 min, holding at 58°C for 1 min, furthermore at 72°C for 1 min, and repeating 30 times. The PCR reaction mixture was fractionated using 1% agarose gel electrophoresis. After staining the gel with ethidium bromide, the ultraviolet irradiation was carried out to detect an amplified band at about 500 bp. Similarly, the glyceraldehyde 3-phosphate dehydrogenase gene amplified primer (G3PDH, Clonetech Co.) was used as the standard cDNA for PCR testing. As a result, the expression of pgth mRNA was strongly induced in the macrophages loaded with oxidized LDL, as shown in Figure 3.

Normal monocytes, macrophages loaded with oxidized LDL or equivalent cultured cells may be cultured with a test compound added, and subsequently the change in the PGTH mRNA may be measured by the method described above to screen any substance controlling PGTH mRNA expression.

Brief description of the figures

Figure 1 shows comparison of amino acid sequence homology between hPGT and the PGTH of this invention.

Figure 2 shows the results of SDS-PAGE of PGTH expressed using the in vitro translation method using pgth.

Figure 3 shows the results of detection of mRNA for the expression of pgth in human macrophages loaded with oxidized LDL using the RT-PCR method. In the figure o shows the results for human macrophages loaded with oxidized LDL, and m shows the results for normal human monocytes.

Claims

(1) A protein of the following (a) or (b).

(a) Protein comprising the amino acid sequence of sequence No. 1

(b) Protein comprising an amino acid sequence with one or more amino acids deleted, substituted or added to the amino acid sequence of sequence No. 1, and, at the same time, having a prostaglandin transport activity.

(2) DNA of the following (a) or (b).

(a) DNA comprising the base sequence of sequence No. 2

(b) DNA which can be hybridized with the DNA of sequence No. 2 under stringent conditions and at the same time, encodes a protein having a prostaglandin transport activity.

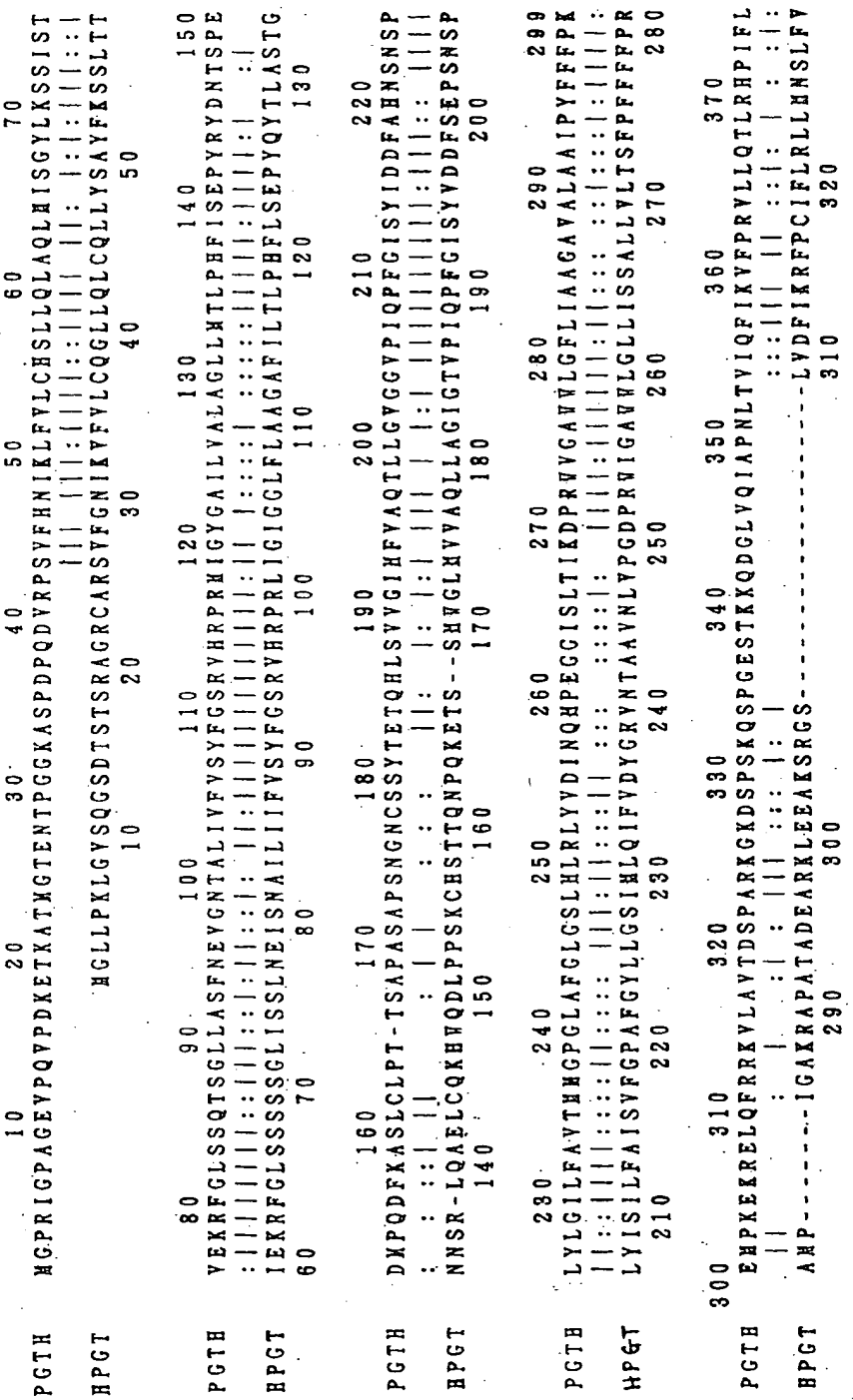

Figure 1

Replacement Sheet (Regulation 26)

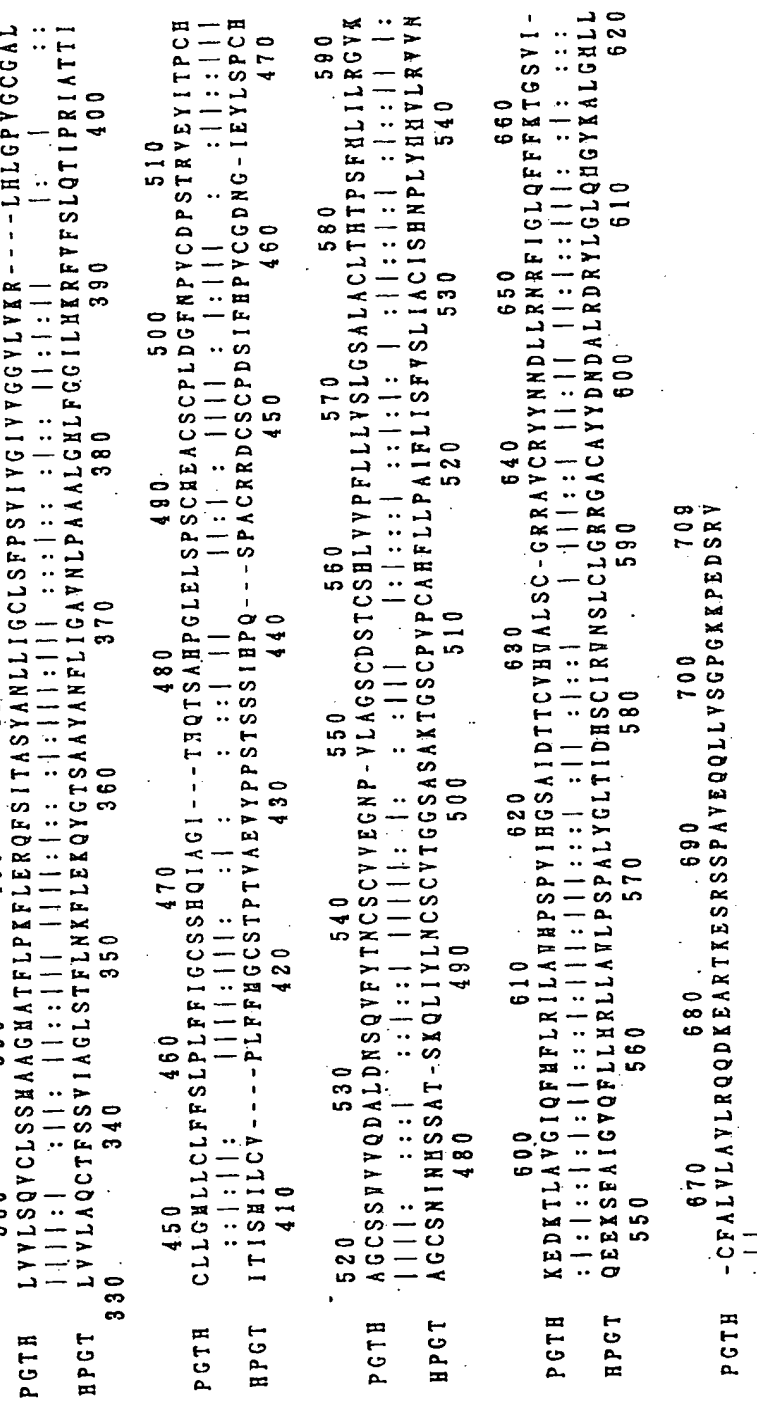

Figure 1 (cont.)

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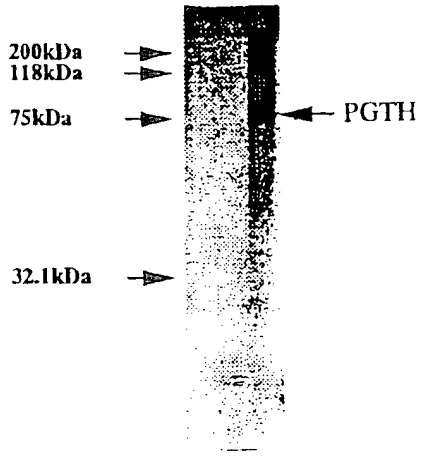

Figure 2

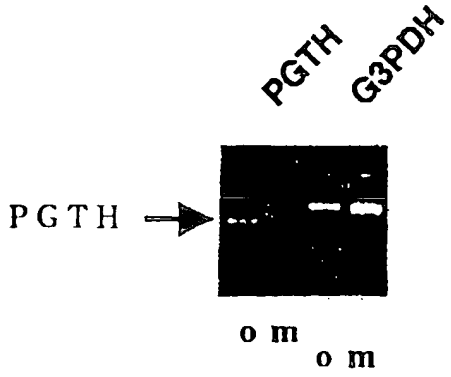

Figure 3

SEQUENCE LISTING

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<150> JP10-227723

<151> 1998-08-12

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<212> PRT
<213> Homo sapience

<400> 1

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35 40 45
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50 55 60
Ala Gln Leu Met Ile Ser Gly Tyr Leu Lys Ser Ser Ile Ser Thr
65 70 75
Val Glu Lys Arg Phe Gly Leu Ser Ser Gln Thr Ser Gly Leu Leu
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His Phe Ile Ser Glu Pro Tyr Arg Tyr Asp Asn Thr Ser Pro Glu
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185 190 195

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Gly Lys Ala Ser Pro Asp Pro Gln Asp Val Arg Pro Ser Val Phe
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/04352

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] C07K 14/47,C12N 15/12//C12N 5/10,C12P 21/02,
(C12P 21/02,C12R 1:91)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] C07K 14/47,C12N 15/12,12N 5/10,C12P 21/02,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
SwissProt/PIR/GeneSeq,Genbank/EMBL/DDBJ/GeneSeq,
WPI(DIALOG),BIOSIS(DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,5792851,A(Albert Einstin College of Medicine of Yeshiva University,a Division of Yeshiva University) 11 August, 1998 (11.08.98) (Family: none)	1,2
Y	Journal of Clinical Investigation, Vol.98, no.5 (1996) Lu Run, et al., "Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA (hPGT)" see p.1142-1149,(1996)	1,2
Y	Biochemical and Biophysical Research Communications, Vol.246, No.3, (May 29,1998), Lu Run, et al., "Molecular cloning of the gene for human prostaglandin transporter hPGT: Gene organization, promoter activity and chromosomal localization", see p.805-812.	1,2

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 November, 1999 (09.11.99)	24 November, 1999 (24.11.99)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/JP99/04352

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, vol.268, No.5212, (1995), Kanai Naoaki et al., "Identification and Characterization of a prostaglandin transporter ", see p.866-869,	1-2